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# Ultraviolet-Light-Absorbing Tunic Cells in Didemnid Ascidians Hosting a Symbiotic Photo-oxygenic Prokaryote, *Prochloron*

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*Coral reef invertebrates that host phototrophic symbionts are thought to protect themselves and their symbionts with mycosporine-like amino acids (MAAs)—UV-absorbing substances that act as sunscreens (Dunlap, W. C., and J. M. Shick, 1998.* J. Phycol. *34: 418–430). However, the histological distribution of MAAs in the host tissues has not yet been visualized. We have localized the UV-absorbing substances in the tissues of two colonial didemnid ascidians—*Lissoclinum patella *and* Diplosoma *sp.—that contain the symbiotic photo-oxygenic prokaryote* Prochloron *sp. Cross-sections of unfixed tissue from these ascidians were examined by UV-light microscopy at 320 or 330 nm, wavelengths at which UV light is absorbed by MAAs. Within the tunic, the gelatinous integument of the colony, UV light was exclusively absorbed by a particular type of cell, the tunic bladder cell. Tunic bladder cells with strong UV absorption were denser in the upper tunic, which lies over a colony's zooids, than in the basal tunic underlying the zooid. In the upper tunic, those cells with strong UV absorption were most dense near the surface. The tunic bladder cell is highly vacuolated, and the vacuole contains strong acid, which destabilizes MAAs. Furthermore, the UV-absorbing portion of tunic bladder cells seemed to be cup-shaped, indicating that the MAAs are not localized in the vacuole, but in the cytoplasm. These results strongly suggest that didemnid ascidians accumulate MAAs in tunic bladder cells as a protection against UV radiation.*

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Ultraviolet (UV) radiation poses severe problems for organisms in tropical marine environments, because the path for sunlight through the atmosphere is shorter (1) and the seawater is clearer (2). Since the discovery of UV-absorbing substances in marine organisms in the Great Barrier Reef (3), it has been thought that many coral reef invertebrates hosting symbiotic microalgae or photo-oxygenic prokaryotes protect themselves and their symbionts by using, as sunscreens, mycosporine-like amino acids (MAAs) which absorb UV (for review see 4, 5, 6).

The MAAs ($\lambda_{max}$ 310–360 nm) have strong absorption in the UVA (320–400 nm) and UVB (280–320 nm) range, and some, porphyra-334 (7) and shinorine (8), have been shown to be photochemically stable to UV irradiation. Under ultraviolet radiation (UVR), they neither fluoresce nor produce radical intermediates (9). The MAAs are transparent to visible light (*i.e.*, photosynthetically active radiation, PAR), which is required by the phototrophic symbionts of many invertebrates whose tissues are also exposed to the UVR.

UVR induces an increase in the MAA content of zooxanthellate corals, microalgae, and seaweeds (for review see 6). Corals and some red macroalgae living in shallow water have greater MAA concentrations than those from deeper or shadowed waters (for review see 4, 6). The photosynthetic ability of symbionts isolated from their host tissues is severely damaged by UVR, whereas that of the symbionts within the host is more resistant (10, 11; for review see 6).

The MAAs are thought to be synthesized by the shikimic acid pathway, which has not been reported in metazoan cells (12, 13; for review see 6). It is thought that the host invertebrates acquire MAAs from their diet or from their symbionts (13; for review see 4). In summary, MAAs, which may be synthesized by the symbiont upon stimulation by UV radiation, function as a sunscreen for the symbionts as well as the host.

In tropical waters, some didemnid ascidians host the symbiotic photo-oxygenic prokaryote *Prochloron* (for review see 14). The symbiont cells are usually sequestered within the host colony under an integument, the ascidian tunic, which is transparent to visible light (PAR), but which absorbs maximally at about 320 nm (10). In a symbiotic didemnid, *Lissoclinum patella*, 93%–98% of UV light (312 nm) was reduced by a slice of its integument tunic, thickness 0.5–1.0 mm, whereas 83%–90% of visible light was passed through the slice (10). The tunic of *L. patella* also contains MAAs, such as shinorine, mycosporine-glycine, and palythine (10, 15, 16). Although the tissue content and chemistry of MAAs have been studied intensively in *L. patella* and a variety of other organisms (5), the detailed distribution of these MAAs in the ascidian tunic—whether they are localized in certain tunic cells or in the tunic matrix—remains to be determined. To approach this problem, we studied the histological distribution of UV-absorbing substances in the tunic of two photo-symbiotic didemnid ascidians hosting *Prochloron;* we used UV-light microscopy at 320 or 330 nm, the wavelengths at which MAAs typically show maximal absorption.

A sheet-like colony of didemnid ascidians usually consists of three layers: a layer of zooids and cloacal cavity (ZC in Fig. 1) sandwiched between two layers of tunic (UT and BT in Fig. 1). Both the upper and basal layers of tunic are transparent to visible light (Fig. 2A; also see fig. 3 in reference 10). In photo-symbiotic didemnid ascidians, the *Prochloron* cells are exclusively distributed within the cloacal cavity, *i.e.*, outside the host tissue (CC in Fig. 1). In one of the host ascidians, *Diplosoma* sp., UV microscopy revealed a strong layer of UV absorbance in the upper tunic and a similar layer with less UV absorption in the basal tunic (Fig. 2B). The strength of the UV absorption seemed to depend upon the density of UV-absorbing objects in the layer. Moreover, the UV absorption of each object seemed to be stronger in the layer of the upper tunic than in the basal tunic (Fig. 2B). Similar UV-absorbing layers were also observed in the other host ascidian studied, *L. patella* (data not shown). These results agree well with a previous report that MAA concentration is higher in the upper tunic than in the basal tunic in *L. patella* (10). *Prochloron* cells occupying the cloacal cavity in the middle layer of *Diplosoma* sp. (ZC layer in Fig. 2) also absorbed UV light. Observation of

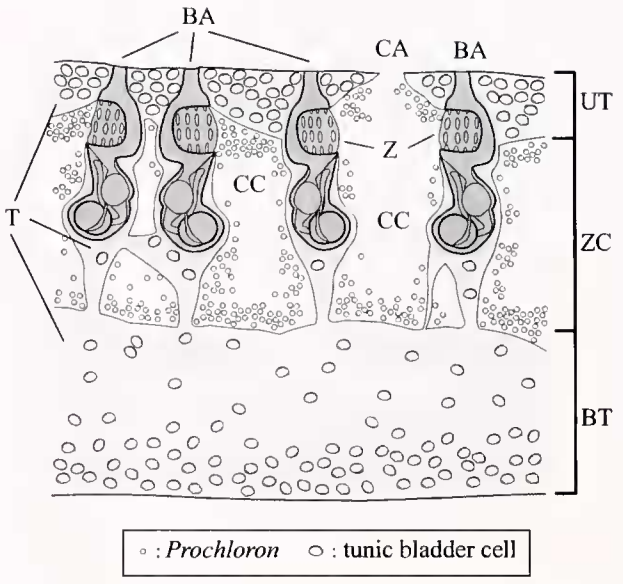


**Figure 1.** Schematic drawing of a cross-section of a colony of a symbiotic didemnid ascidian. BA, branchial aperture; BT, basal tunic layer; CA, cloacal aperture; CC, cloacal cavity; T, tunic (lightly shaded area); UT, upper tunic layer; Z, zooid (more darkly shaded area); ZC, layer of zooid and cloacal cavity.

the upper tunic at higher magnification revealed that each UV-absorbing object was round or cup-shaped, with a diameter (of the openings of the cup) of 30–43 $\mu$m (Fig. 3). When cross-sections of fixed, embedded, and stained ascidian tissues were observed, the tunic was seen to contain many bladder cells, about 50 $\mu$m in diameter; moreover, these cells correspond to the UV-absorbing objects (Fig. 4A). This obviously indicates that the MAAs are not localized in the matrix of the tunic, but in the tunic bladder cells. The bladder cell is characterized by a large vacuole occupying most of the cytoplasm (Fig. 4B). Such bladder cells are common in the tunic of many ascidians in the family Didemnidae (17), and their vacuoles usually contain a strongly acidic fluid (18). The cup shape of the UV-absorbing objects (Fig. 3) indicates that the UV-absorbing substance is contained in the cytoplasm of the bladder cell, and that the large vacuole lacks this substance. Because the vacuoles of the bladder cell are known to contain strong acid (17, 18), and because MAAs are unstable in acidic conditions (19), it is reasonable that these MAAs are localized in the cytoplasm.

We have previously reported that the tunic of *L. patella* contains shinorine, one of the most common MAAs, as a major UV-absorbing substance, together with two minor MAAs, mycosporine-glycine and palythine (10); in Australia, however, *L. patella* was reported to contain shinorine and mycosporine-glycine (15), or only mycosporine-glycine (16). In this study, we freeze-dried *Diplosoma* sp., extracted the material with a methanol-tetrahydrofuran (4:1) mixture,

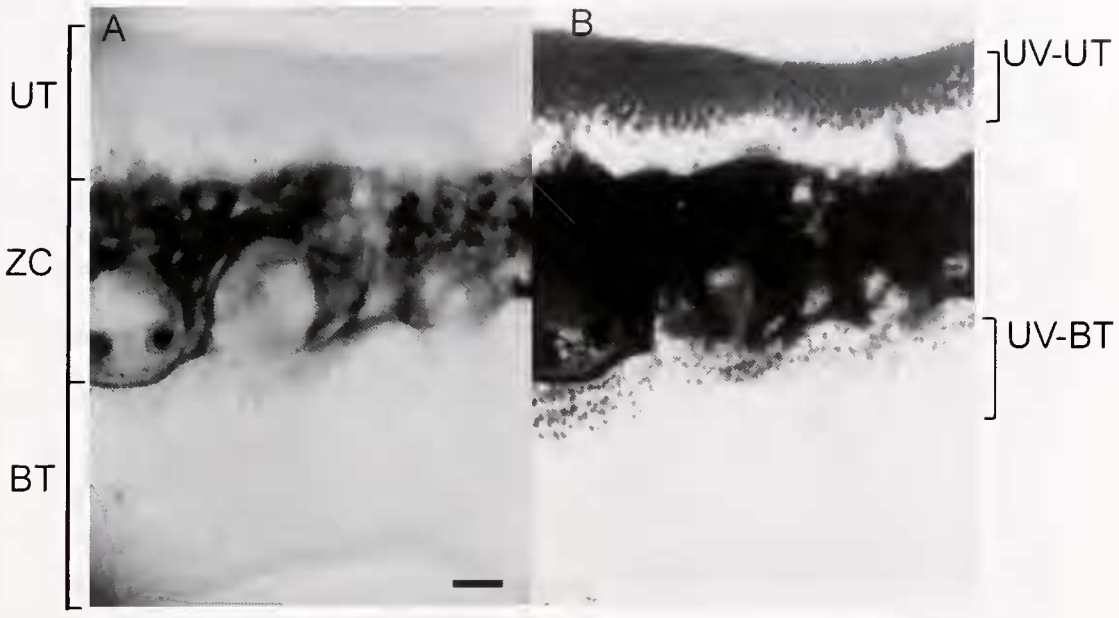


**Figure 2.** Visible- and ultraviolet-light micrographs of a cross-section of a living colonial ascidian, *Diplosoma* sp. The ascidian sample was collected at Akajima, an island in the Ryukyu Archipelago, Japan. Thin slices of living specimens were cut with a razorblade by hand. They were placed on a quartz slide and immersed in filtered seawater surrounded by glycerol, covered with a quartz coverslip, and observed under a light microscope with Nikon Fluor objectives (10× NA = 0.5, 20× NA = 0.75). The glass eyepiece and condenser lens were removed, because they absorb UV light. An interference filter (bandpass) for 319 ± 11 nm (median ± 50% maximum transparency) or for 331 ± 5 nm (median ± 50% maximum transparency) was inserted between a reflex camera body (Olympus OM-2) mounted on the microscope and the objective lens. The light source was a UV lamp (Vilber-Lourmat T15-N, France; or Toshiba F6T5 UV-B lamp, Japan). Black-and-white film (Fuji Neopan F) was used to record the images. Because the focal plane for the UV light is different from that for visible light, it was determined before the experiment by making test exposures. For visible-light microscopy, the interference UV bandpass filter was removed from the light path, and an UV-opaque filter was inserted between the light source and the specimen. (A) Visible-light micrograph; (B) UV-light micrograph (320 nm). UT, upper tunic layer; BT, basal tunic layer; ZC, zooid and cloacal cavity layer; UV-UT, UV-light-absorbing zone in the upper tunic; UV-BT, UV-light-absorbing zone in the basal tunic. Scale, 200 μm.

and analyzed the extracts for MAAs by reversed-phase liquid chromatography according to Dionosio-Sese *et al.* (10); the MAAs from another ascidian, *Halocynthia roretzi*, were used as references (20). The major MAA (about 94%) was shinorine, and the minor MAA was palythine. Because these substances are soluble in water (21), MAAs probably exist as soluble components in the cytoplasm of the bladder cells. Isolated *Prochloron* cells from *L. patella* contained shinorine, which was also dominant in the host tunic (10). MAAs are thought to be synthesized by the shikimic acid pathway, which has not been reported in metazoans cells (12, 13). In symbiotic didemnid ascidians, the MAAs are probably synthesized in the symbiont algal cells, then transferred to the host tissue, and accumulated in the cytoplasm of the bladder cells mostly in the upper tunic.

Because the UV-absorbing layer in the upper tunic is transparent to PAR but absorbs UVR, the underlying host tissues and the symbionts are protected from the UVR, but they can still receive PAR needed for photosynthesis. The

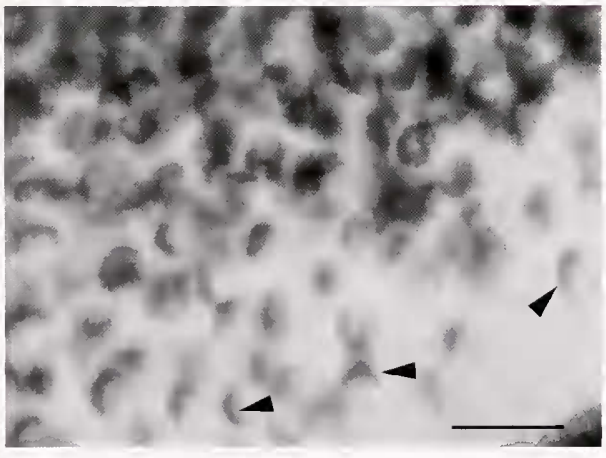

**Figure 3.** Higher magnification UV micrograph (320 nm): cross-section of a UV-absorbing zone in the upper tunic of a living colony of *Diplosoma* sp. The cytoplasm of tunic bladder cells was observed as dark, round or cup-shaped, UV-absorbing objects (arrowheads). Scale, 100 μm. For UV microscopy, see the legend of Figure 2.

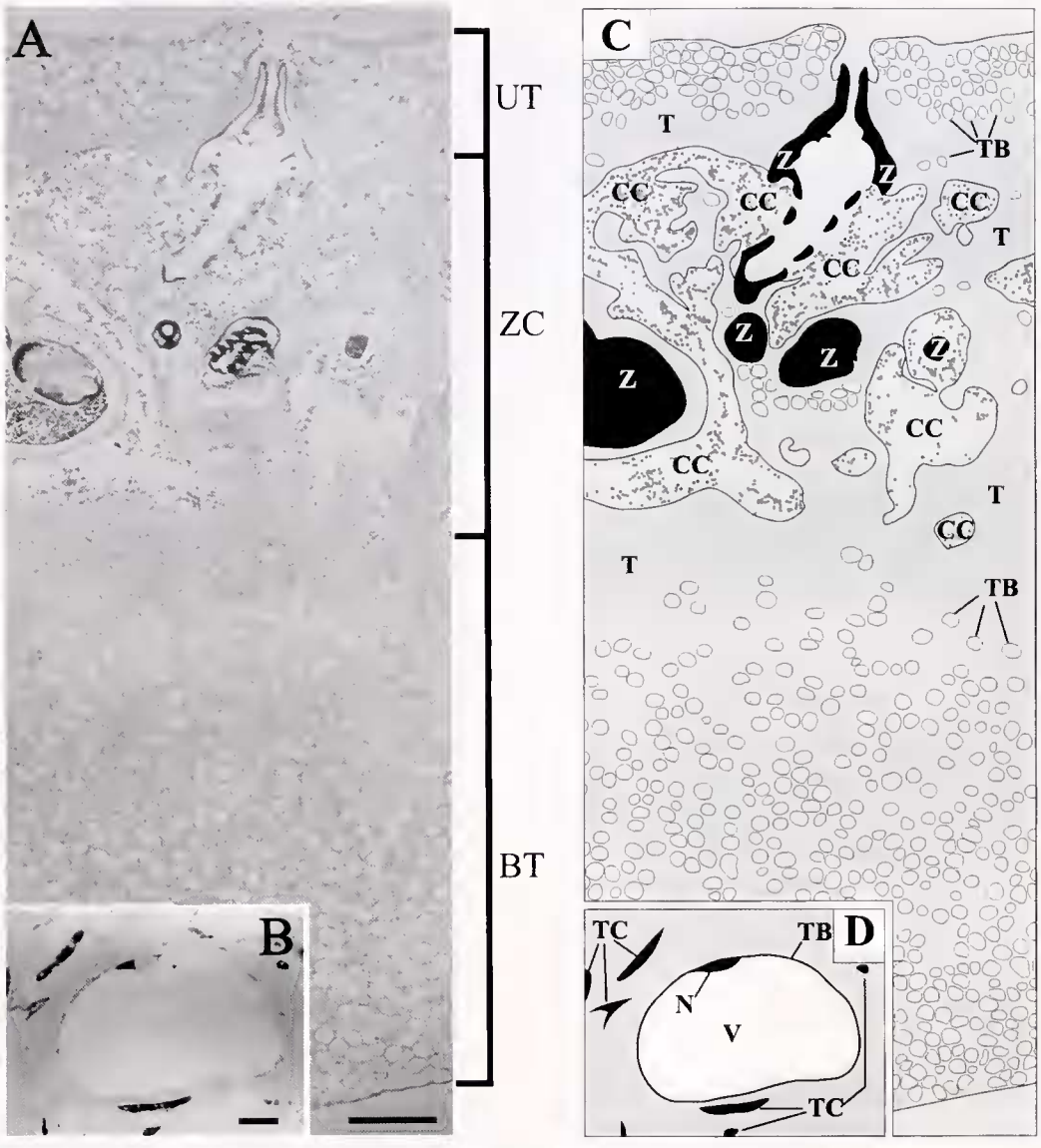


**Figure 4.** (A) Light micrograph of a cross-section of a colony of *Diplosoma* sp. (10% formalin-seawater fixation; 10-$\mu$m-thick paraffin section stained with hematoxylin and eosin). Scale, 200 $\mu$m. (B) Higher magnification light micrograph of a tunic bladder cell (2.5% glutaraldehyde prefixation and 1% $OsO_4$ postfixation; 1-$\mu$m-thick resin section stained with toluidine blue). Scale, 10 $\mu$m. (C and D) Schematic representations of A and B, respectively. CC, cloacal cavity; N, nucleus of tunic bladder cell; TB, tunic bladder cell; TC, other tunic cells; V, vacuole of tunic bladder cell; Z, zooid; small structures (dots) in CC, cells of *Prochloron* sp.

mechanisms underlying translocation of MAAs and their uptake by the tunic bladder cells remain to be elucidated.

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